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CONTROL OF EGG HATCHING IN THE CRAB *RHITHROPANOPEUS HARRISII* (GOULD)

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Abstract

Ovigerous females of the crab *Rhithropanopeus harrisii* were collected from an estuary having irregular tides. When monitored under constant conditions in the laboratory, the crabs have a circadian rhythm in larval release. Eggs removed from the female within 2 days of hatching hatched at about the same time as larvae were released by the female. Hatching became increasingly desynchronized with longer removal times. Upon exposure to water in which the larvae hatched, ovigerous females diplayed increased abdomen pumping, a behavior observed at the time of larval release. The active substance was released at the time of egg hatching but not by newly hatched larvae. Homogenized eggs of different ages and homogenized larvae induced similar behavior. There was no change in female sensitivity with clutch age or time of day. Active pumping by the female only induced hatching at times predicted by the larval release rhythm, not at other times during the solar day. These results indicate that an interaction between the eggs and female is responsible for synchronized development while the actual timing of hatching is controlled by the embryo. At this time an active substance is released. This substance induces abdomen pumping by the female which serves to synchronize larval release.

Introduction

Precisely-timed rhythms in larval release are common among crustaceans. The timing may be related to lunar phase (Christy, 1978, 1982; Saigusa and Hidaka, 1978; Wheeler, 1978; Saigusa, 1981), time of day (Ennis, 1973, 1975; Branford, 1978; Moller and Branford, 1979), or phase of the tide (DeCoursey, 1979; Bergin, 1981). Detailed studies of fiddler crabs (DeCoursey, 1979) and lobsters (Ennis, 1973) indicate that larval release lasts only a few minutes and is associated with rapid movements of the ovigerous female's abdomen and pleopods. Since larval release is a short, precisely-timed event, an important question is whether the timing is controlled by the female or the developing embryos.

Previous studies produced divergent results. DeCoursey's (1979) work with the fiddler crab *Uca minax* suggests that physical stimulation of hatching by the female is necessary for larval release. For the lobster *Homarus gammarus,* Branford's (1978) results indicate that larval release is regulated by the female and that her role in the hatching process is under endogenous control. Alternatively, other investigators (Pandian, 1970; Ennis, 1973) have suggested that for this lobster species the clock which sets the hatching time is in the egg itself.

The estuarine crab *Rhithropanopeus harrisii* also releases its larvae over a short time interval (Forward *et al.,* 1982). In the laboratory under constant conditions, larval release by crabs from an estuary lacking regular tides occurs mainly in the

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2 h interval after the time of sunset, which suggests the presence of a circadian rhythm. In contrast, releases in the laboratory by crabs from an estuary with semidiurnal tides generally begin at the time of high tide in the field and continue for 2 h, suggesting a circatidal rhythm. The present study examines whether the female or the embryo is controlling the timing of hatching.

Using crabs with a circadian rhythm, we first determined whether the detached eggs hatch at the same time as eggs attached to the female. Since the timing was similar, we then experimented to determine if a chemical cue from the hatching eggs induced the female to undergo the behavioral sequence observed during larval release.

Materials and Methods

The crab *Rhithropanopeus harrisii* (Gould) was collected from the Neuse River estuary (North Carolina). Ovigerous females were obtained either from the field or from a breeding population in a laboratory habitat (described in Forward *et al.*, 1982). Females were maintained in an environmental chamber (Sherer Gillett Co., Model CEL 4-4) at 26–27°C in 8 ppt sea water which was filtered to remove particles larger than 5 μ. A 14 h light:10 h dark photoperiod was employed. Under these conditions, females from both the field and the laboratory habitat release larvae in the interval beginning at the end of the light phase and concluding about 2 h later (Forward *et al.*, 1982). The general experimental procedures are described below while specific modifications are explained in the Results section. The term "eggs" refers to the combination of outer covering, enclosed non-living material, and the developing embryo before hatching.

The first series of experiments was designed to determine whether detached eggs can hatch and if so, whether the time of hatching is similar to that of larval release by the female. Hatching was monitored under 3 conditions: detached eggs in still water, detached eggs on a mechanical shaker, and eggs attached to the female. Crabs with eggs which would hatch within 1 day (based on eye development and yolk consumption) were used. For monitoring hatching by detached eggs in still water 100–200 eggs were removed from each female about 3 h before the end of the light phase and placed in a 7.9 cm diameter finger bowl containing 8 ppt sea water filtered to remove particles larger than 5 μ. Preliminary observations indicated that most of the eggs hatched in a several hour interval just after the beginning of the dark phase. To quantify this sequence, hatching was monitored over a 5 h sampling time beginning 1 h before the end of the light phase. At 0.5 h intervals, the number of free swimming zoeae was counted and removed by pipette from the finger bowl. Care was taken not to swirl the bowl water during removal. After the day phase ended the eggs were placed in constant low level light (photographic safe light containing a 15 W bulb and fitted with a Kodak OA filter; wavelength maximum = 573 nm, half band pass 37 nm, intensity = 10^{-2} W/m^2) at 27° ± 1°C. A microscope illumination lamp (American Optical Co.) interference filtered to 660 nm (Ditric Optics Inc., half band pass 11 nm) was used briefly to see the larvae each half hour. Larvae are very insensitive to this wavelength (Forward and Cronin, 1979). If eggs remained at the end of the 5 h sampling interval, then they were maintained under constant conditions, and the observation procedure was repeated at the next monitoring time, 19 h later.

In order to simulate the pumping action of the female a similar group of eggs was placed in a finger bowl on a mechanical shaker (Eberbach Corp.). Eggs were shaken at a rate of about 1 cycle/s. Shaking began 1 h before the beginning of the

dark phase and continued until hatching occurred. Thus eggs which hatched at the time of the second night in constant conditions had actually been shaken continuously for at least the preceding 24 h. The bowl was briefly removed from the shaker to count swimming zoeae.

Simultaneously, we also determined the time of larval release by the parent female with attached eggs. Crabs were subjected to the same conditions as the eggs. During the 5 h sampling interval, the female was transferred every 30 min to a new 7.9 cm diameter finger bowl. At the end of the sampling period, she was placed in a 10.4 cm diameter finger bowl and if eggs remained, the procedure was repeated at the next monitoring time. The number of larvae released within each 30 min interval was recorded. Crabs and detached eggs were monitored only for 2 consecutive nights in constant conditions.

The detached eggs hatched over several hours (see Figs. 1A and 1C for typical profiles). For the females with eggs, most larvae are released within a 30 min interval, though a few commonly appear in the intervals immediately preceding and following the peak (Fig. 1B). The mean time of hatching by the eggs and larval release by the female was calculated by multiplying the number of larvae observed in each 30 min interval by that interval, taking the sum of these products over all intervals and dividing this sum by the total number of larvae. In this way a single 30 min interval was designated as the time of hatching/larval release.

The next series of experiments was designed to test for the presence of chemical communication between the eggs and the female. At the time of larval release the female elevates her body upon her walking legs, then repeatedly flexes her abdomen. Larvae are released with each "pump."

The frequency of abdomen pumping was used to quantify the inclination of the female to undergo larval release behavior. The procedure was to first place the female in a 7.9 cm diameter finger bowl containing 40 ml of 8 ppt sea water filtered to remove particles larger than 0.2 μ and at 27 $\pm$ 1°C. The number of pumps in the initial 2 min interval was counted by a stationary observer. The crab was then placed in 40 ml of the test solution (*e.g.*, water in which hatching had occurred) and the number of pumps in the initial 2 min period was similarly recorded. The control consisted of placing previously untested crabs sequentially in clean 8 ppt sea water. The control level did not change with embryo development and is reported for females with eggs that would hatch within 3 days. Each crab was used only once in a particular test solution. Initial experiments were conducted during the 5 h interval after the beginning of the dark phase, because this is the time of normal larval release. In this case the crabs were observed under red light. Since we subsequently found that responsiveness by the crabs does not vary over the day, later trials were conducted under room lighting during the day.

Results

Hatching by separated eggs

A typical hatching profile of eggs removed from the female and kept in still water is shown in Figure 1A. Hatching of detached eggs is not as synchronized as larval release by the female (Fig. 1B). It usually begins shortly before the time of greatest release by the female and continues over about the next 3 to 4 hours.

Most of the crabs (95%) both released their larvae and had their detached eggs hatch shortly after the beginning of the night phase. For all of the eggs monitored in still water (Fig. 2), an average of 96% (SE = 0.8%) of each group hatched within the 5 h sampling interval. These results clearly indicate hatching can occur inde-

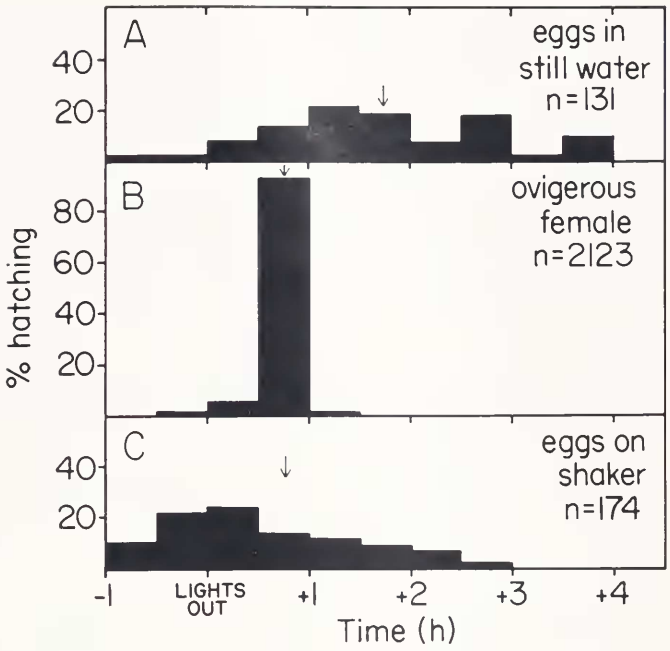

FIGURE 1. The percentage of eggs hatching (ordinate) from one crab over time (abscissa) as related to the time of the normal end of the light phase (lights out). The eggs in still water (A), left upon the ovigerous crab (B), and eggs shaken continuously (C), were placed under constant conditions about 24 h before hatching. The arrows indicate the mean time of hatching. n is the number of hatched eggs.

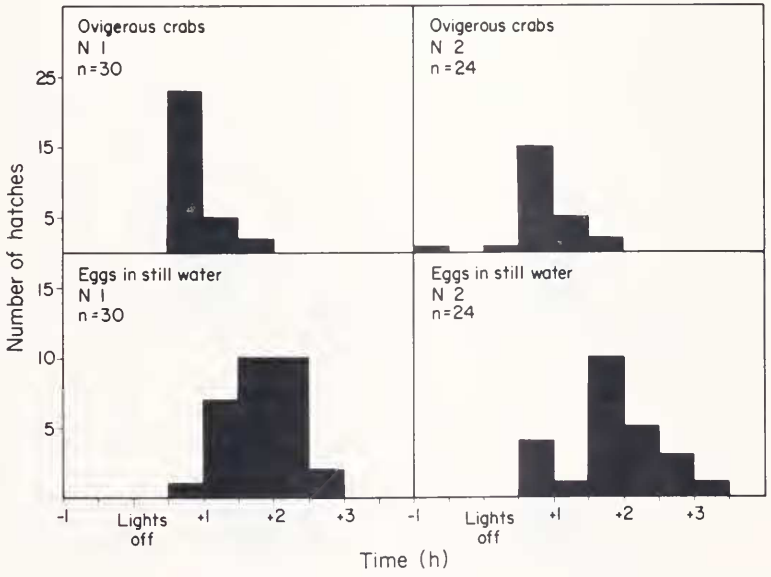

FIGURE 2. Distribution of mean times of larval release by ovigerous females and of hatching by their detached eggs for different numbers of crabs (ordinate) relative to time (abscissa) of end of the light phase. After "lights off" on the first night (N-1), crabs and separated eggs were maintained in constant conditions and monitored again at the time of the next night (N-2). n indicates the number of hatches or releases measured for each condition.

pendently of the female and is not randomly occurring over the solar day. Thus it is possible to compare the mean time of hatching by detached egg to the mean time of larval release by the female. On all nights and conditions (Fig. 2) hatching times are not uniformly distributed over the 5 h sampling interval ($P < .05$, Kolmogorov-Smirnov test for goodness of fit). The greatest number of females released larvae between 30–60 min after the beginning of the dark phase, while for the detached eggs the time is about 1 h later (Fig. 2). This relationship was further verified by specifically comparing the mean hatch time of the detached eggs to the mean time of larval release by the parent female. The modal time of hatching by detached eggs was 1 h later. In conclusion: eggs detached within 2 days of hatching hatch at about the same time as those attached to the female, but the former do so more variably and about 1 h later.

The differences between the two situations may result from the females' behavior, that is, vigorous female pumping may assist the opening of the egg covering, resulting in the release of most of the larvae over a shorter period of time. To test this hypothesis the previous experiment was expanded to include a group of detached eggs which were placed on a mechanical shaker. The eggs were shaken continuously to determine whether mechanical agitation alone causes hatching at times other than the interval just after the end of the day phase. The female does not pump her abdomen continuously.

A typical hatching sequence for this group is shown in Figure 1C. Hatching occurred earlier in the night, as compared to eggs in still water (Fig. 1A). However, larval release by the female was still more synchronized. Most of the crabs (91%) both released their larvae and had their detached eggs on the shaker hatch shortly after the end of the day phase. For these detached eggs (Fig. 3), an average of 99% (SE = 0.4%) of each group hatched within the 5 h sampling interval. Therefore, hatching is not occurring randomly and agitation assists hatching only during the time interval at the beginning of the dark phase.

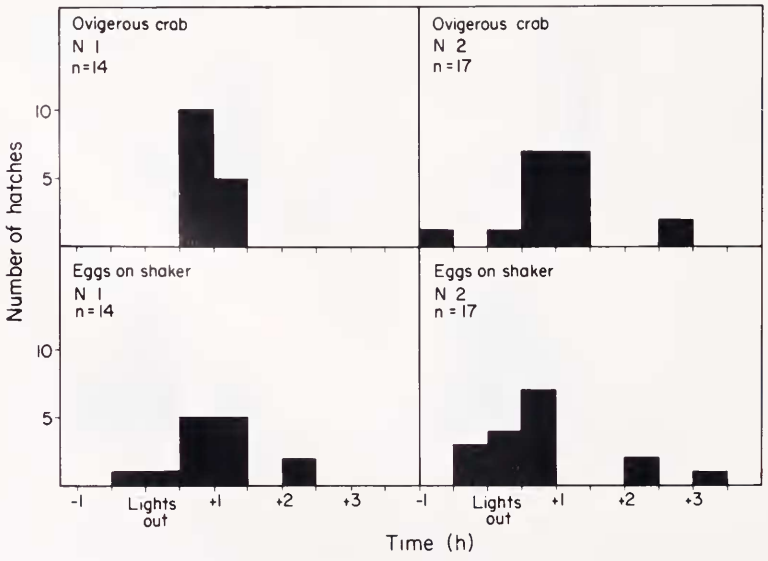

FIGURE 3. Distribution of larval releases and of hatching by eggs on shaker relative to the end of the light phase. Symbols, as in Figure 2.

On all nights and conditions (Fig. 3), hatching times are not uniformly distributed over the 5 h sampling interval ($P < .05$). In general, hatching by detached eggs on the shaker occurs near the times of larval release by the females (Fig. 3). If the mean hatching time of the shaken eggs is compared to the mean time of larval release by the parent female, the modal difference in timing is zero (n = 30). Thus the shaken eggs hatch at about the same time as eggs attached to the female, and mechanical agitation seems to mimic abdomen pumping.

For all but two crabs, larval release occurred on a single night. In the two exceptions the crabs released one group of larvae on the first night and the rest at the time of the second night. This is not unusual (Forward *et al.*, 1982). For these crabs, detached eggs were also monitored in still water and on the shaker. In both cases some of the eggs hatched on the first night with the remaining eggs hatching about 24 h later. These results further support the conclusion that hatching in the detached eggs occurs at about the same time as larval release by the female.

Although this conclusion is consistent for eggs removed within 2 days of hatching, a further question is whether eggs, which are removed from the female earlier, hatch in synchrony with eggs attached to the female. We selected 3 crabs with eggs which would hatch in about 9 days and entrained them for 4 days to a 14 h light:10 h dark photoperiod having the beginning of the dark phase at 1200. The time of end of the day phase was shifted so that hatching would occur at a convenient time. Four days is sufficient to shift the timing of the rhythm (Forward *et al.*, 1982). Beginning 5 days before the expected time of hatching, a group of approximately the same number of eggs (average difference = 26%) was removed from each crab at daily intervals. The eggs and female were maintained in aerated 8 ppt sea water filtered to remove particles larger than 0.2 μ and to which the antibiotic chloramphenicol was added (5 mg/l). Extremely clean water containing the antibiotic was necessary to permit viable embryo development. Only 1% of the removed embryos died. Chloramphenicol at this concentration does not affect biological rhythms in eukaryotes (*e.g.*, Goodenough *et al.*, 1981). The water was changed every other day. The eggs and female were maintained at 27° ± 1°C on the LD cycle throughout the experiment. On the day when hatching was expected, larval release by the females and hatching by the free eggs was monitored in still water at 1 h intervals beginning 2 h before the end of the light phase.

The hatching cycles are shown in Figure 4. Since the number of eggs removed each day from each female was similar, the total numbers of eggs hatched in all 3 broods were combined, and the absolute numbers presented for each 1 h interval. For the ovigerous females the number of released larvae differed greatly. To combine these data, the percentages of larvae released in each 1 h interval were averaged. Eggs removed on the final day of development hatched at about the same time as larvae were released by the females. For eggs removed for longer times, hatching was never uniform ($P < .05$, Kolmogorov-Smirnov test for goodness of fit) over the 32 hours that hatching was monitored. However, hatching became increasingly desynchronized with longer removal times. Since the eggs were maintained on the LD cycle, synchrony does not result only from entrainment on a LD cycle. This result suggests that some aspect of the female-egg interaction is important in establishing synchronized hatching.

Cues from the eggs

The next series of experiments was designed to answer several questions. First, is there a chemical cue released at the time of hatching which induces pumping

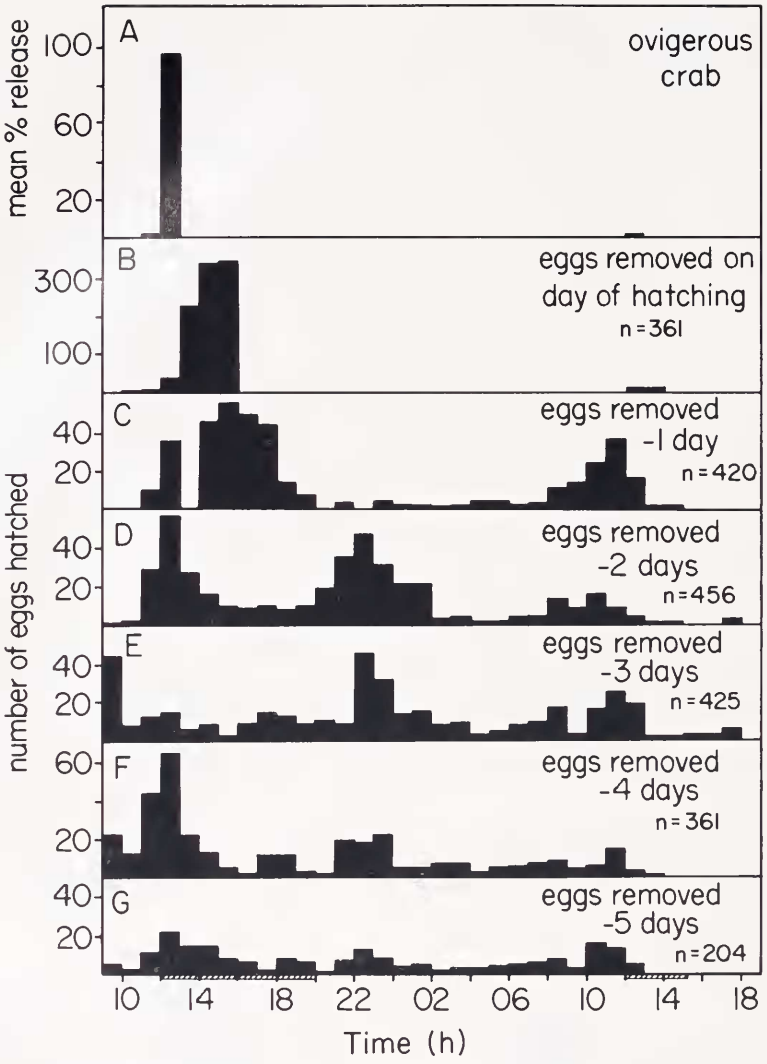

FIGURE 4. Distribution of larval releases by 3 females (A) and of hatching of their detached eggs (B-G) relative to time in the LD cycle. Eggs from only 2 crabs were used for G. The cross hatched bars indicate the time of the dark phase. In all cases greater than 94% of the eggs hatched during the observation time. n indicates the number of eggs hatched for the different conditions.

behavior by ovigerous females? If so, does receptivity to the cue change with age of the female's embryos?

After a female released her larvae into a volume of clean water (filtered initially to 0.2 μ), she was quickly removed. The water was filtered to remove the larvae, and the larvae were counted. The water was diluted so that there was 1 ml for each 40 larvae released. This concentration of "larval water" was selected because pilot experiments showed that it induced a strong pumping response (Fig. 5). Upon exposure to this larval water, ovigerous females showed an initial period of agitated movement (0.25 to 1.5 min), after which they elevated their bodies on the walking

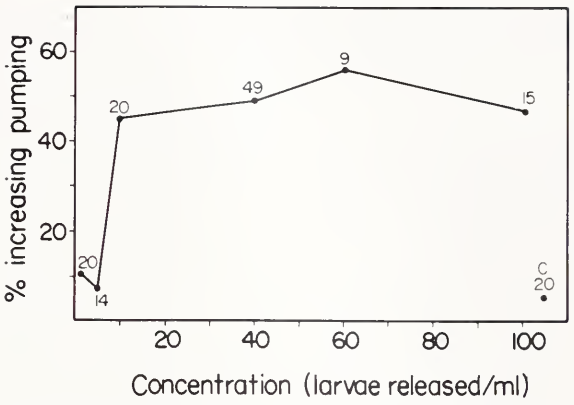

FIGURE 5. The percentage of crabs increasing their pumping rate (ordinate) upon exposure to water in which different concentrations of larvae hatched (abscissa). The number near each point is the total number of crabs tested at that concentration. C is the control and indicates the percentage of crabs increasing their pumping rate upon sequential exposure to clean 8 ppt sea water.

legs and vigorously pumped their abdomens. Pumping by ovigerous crabs with different age embryos (determined by eye development and yolk consumption) was monitored first in clean water and then in the larval water.

The percentage of crabs which showed an increase in pumping rate in the larval water was significantly greater ($P < .05$, Z statistic for comparing proportions) for all ovigerous crabs, as compared to non-ovigerous females (Table I). There was also a significant increase in the mean pumping rate (Student's t test, $P < .05$) when exposed to larval water. However, the rates were not significantly different between crabs with different age embryos (One-way ANOVA, model I).

These results indicate that the water in which the larvae were released contained a chemical which induced behavior observed during larval release by the female. In addition, even though the largest response occurred among crabs with the oldest embryos, there was little change in responsiveness throughout embryonic devel-

TABLE I

Variation in female pumping response with embryonic development

Estimated time until egg hatching (days)	n	% crabs increasing pumping	Number of pumps/2 min			
			clean water		hatch water	
			m	SE	m	SE
0–1	18	61	1.1	0.5	8.3	2.1
2–3	31	42	1.5	0.5	4.5	1.4
4–5	19	53	1.2	0.5	4.6	1.4
6–7	33	33	0.6	0.3	3.6	1.4
>7	35	46	0.4	0.2	5.4	1.4
NOF	22	14	1.2	1.2	2.5	1.5

The percentages of crabs displaying an increase in pumping in the hatch water as compared to pumping in clean water and the mean (m) number of pumps/crab are shown. NOF indicates non-ovigerous females. n is the sample size and SE is standard error.

opment. For uniformity in future experiments, tests were run with crabs having embryos which were expected to hatch within 3 days.

To test for a change in female responsiveness to different concentrations of the chemical cue, the water in which the larvae were released was diluted to a range of concentrations (Fig. 5). Responsiveness varied with concentration. Concentrations of 1 and 5 larvae/ml induced responses indistinguishable from the controls. Responses to concentrations of 10 larvae/ml or higher were significantly greater ($P < .01$) but were not significantly different from another.

Is the chemical cue released at the time of egg hatching or is it emitted by newly hatched larvae? To answer this question larvae, immediately upon hatching, were twice transferred to clean water in finger bowls. This served to wash them and dilute any chemical cue in the hatch water from which the larvae were transferred. The larvae were then placed in clean water (concentration 40 larvae/ml) for 2 h, then removed by filtration through clean plankton netting. This water was tested against clean water for its ability to induce increased pumping by ovigerous crabs. Only 15% (n = 20) of the crabs tested showed an increase in pumping rate in the larval water. The expected response at this concentration is 49% (Fig. 5), which is significantly greater ($P < .02$). Furthermore the per cent response is not significantly different from that of control crabs tested in clean sea water (5% response; n = 20). The results indicate that the active chemical is not emitted by the larvae but rather is released at the time of egg hatching.

To learn if the response can be elicited by crushed eggs, and if so, whether there is a difference in effectiveness with embryo age, we removed eggs which would hatch within 1 day (oldest embyros) or within about 8–10 days (youngest embryos). The eggs were homogenized in clean water, and the homogenate was then diluted to the appropriate concentration. There was an increase in pumping response with egg concentration (Fig. 6). The percent response was significantly greater ($P < .05$) than

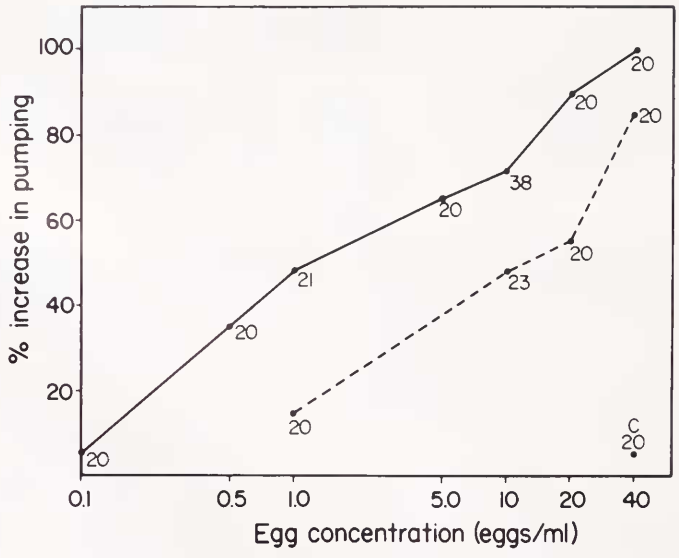

FIGURE 6. The percentage of crabs increasing their pumping rate (ordinate) upon exposure to different concentrations of homogenized eggs (abscissa). Solid line, responses to eggs expected to hatch within 1 day; dashed line, responses to eggs expected to hatch in 7 or more days. Numbers of females tested are shown adjacent to each point. C, controls tested sequentially with clean (8 ppt) sea water.

the control level at all but the lowest test concentrations (0.1 egg/ml older embyro; 1.0 egg/ml young embryo). At all concentrations the crabs were more responsive to the older embryos ($P < .04$), which indicates that the amount of the active chemical increases with embryonic age.

The potency of the crushed older eggs may be a cumulative result of the embryo, its embryonic fluid, and egg membrane. This suggestion is supported by two observations. First, the levels of response to different concentrations of the larval water (Fig. 5) were below levels shown in response to comparable concentrations of crushed eggs (Fig. 6). Secondly, if newly hatched zoeae were homogenized and the resulting mixture diluted to a concentration of 10 larvae/ml, the response level was 40% ($n = 25$), which is significantly ($P < .03$) lower than that for 10 eggs/ml (67%; Fig. 6). Thus, the egg parts produce responses which were below those of the eggs themselves.

Is there a rhythm in female responsiveness to the chemical cue over the day? This was tested by collecting crabs expected to release larvae in 2 days and maintaining them on a 14 h light:10 h dark cycle in phase with field LD cycle. Starting the morning after collection, the crabs were tested every 4 h in clean water and then in a solution having a concentration of 10 homogenized eggs/ml. The test solution was prepared from eggs which would probably hatch within 1 day. This concentration was used because it induces a substantial but not a maximal response (Fig. 6). Crabs were maintained in clean water between trials. At night, pumping was monitored under red light. Preliminary tests indicated that the test solution retained its activity for at least 36 h if refrigerated. Therefore a stock solution was prepared. A 40 ml aliquot was removed from the refrigerator 3 h before each test and allowed to warm to experimental temperatures (27°C). Responsiveness did not change over the day (Fig. 7), as neither the percentage of crabs showing an increase in pumping nor the average pumping rates varied significantly.

To test if induced female pumping can cause the eggs to hatch, we maintained crabs on a 14 h light:10 h dark cycle in the laboratory until the expected day of larval release. Crabs were then tested at 0.5 h intervals beginning shortly before the predicted time of larval release. These crabs were sequentially tested in clean water and in a solution having a concentration of either 20 or 40 homogenized eggs/ml. The eggs were predicted to hatch in 1 day. These concentrations were used because they induce strong responses (Fig. 6). After testing, crabs were rinsed in clean water and then returned to a new holding bowl also containing clean water. The number of pumps in 2 min for each trial and the number of larvae released into each bowl were recorded.

Even though the egg solution induced strong repetitive pumping in all tests, in no case did this action cause an early mass release of the larvae (Table II). Only an occasional larva appeared. This result indicates that egg hatching is not induced by vigorous pumping, except at times when the larvae are predicted to hatch.

Discussion

The embryos of *R. harrisii* can complete development and hatch as viable larvae even when removed from the parent female. If eggs are removed within 2 days of hatching, they hatch at approximately the same time as larval release by the females. Forward *et al.* (1982) showed that crabs maintained under the constant conditions used for the present study have a circadian rhythm in larval release. The similarity in the timing suggests that the detached eggs also have a circadian rhythm. The conditions of the experiment, however, do not fully meet the requirements for demonstrating the presence of a circadian rhythm. The cycle in hatching was only

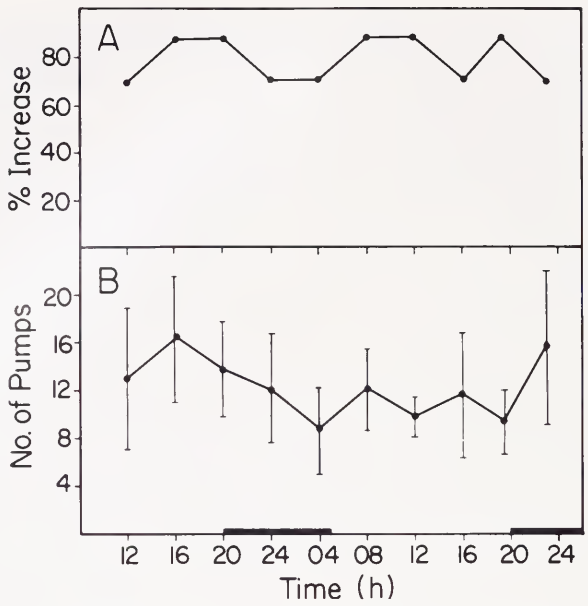

FIGURE 7. The percentage of crabs (N = 8, used throughout) increasing their pumping rate (A) and the average number of pumps/2 min for all tested crabs (B) upon exposure to a solution having a concentration of 10 crushed eggs/ml at different times of day (abscissa). Brackets are SE. The time of the dark phase is indicated by the heavy black bar.

measured in eggs maintained under constant conditions for the last two days of embryo development, and hatching time was measured as the mean time for a population of eggs from one female. The requirements for the endogenous rhythm to persist for 5–10 cycles in a single individual under constant conditions cannot be fulfilled. Thus the results only suggest the presence of a circadian rhythm in the detached eggs.

The results with *R. harrisii* eggs differ from those of Branford (1978) for hatching in the lobster *Homarus gammarus*. In that case detached eggs hatched rhythmically under a LD cycle but arrhythmically in constant light or darkness. His procedure may contribute to these results, since the eggs were removed and held under constant conditions for 3 days before hatching was monitored. Eggs removed from *R. harrisii* for longer than 2 days (Fig. 4) become progressively desynchronized in their hatching, even when exposed to a LD cycle.

Ovigerous *R. harrisii* show rhythmic larval releases after up to 5 days in constant conditions (Forward *et al.*, 1982). The difference between the persistence of the larval release rhythm by ovigerous females in constant conditions and the loss of hatching synchrony by detached eggs in a LD cycle (Fig. 4) suggests that some unknown aspect of the maternal environment is responsible for the establishment or maintenance of developmental synchrony, apparent during the last 2 days of embryonic development.

Although detached eggs hatched in still water, they were not as synchronized as the larval release by the female (Fig. 1). The rapid, vigorous pumping of the females' abdomen during hatching must enhance synchrony, since groups of larvae are released with each pump. This suggestion is supported by data (Figs. 1, 3) showing that hatching synchrony in detached eggs was improved by shaking.

Since pumping improves synchrony it is important to know whether egg hatching

induces pumping. Females placed in water in which larval release occurred showed an increase in pumping. This indicates chemical and not mechanical cues are involved. For *R. harrisii,* the active chemical is apparently released at the time of egg hatching, since newly hatched larvae did not excrete a substance which induced pumping. Responsiveness is confined to females carrying eggs, as nonovigerous females showed a very low level of response. These nonovigerous females were newly collected, and it was possible that the few responsive individuals had either recently released larvae or were about to oviposit.

Responsiveness did not vary in the female. There was no diel rhythm or change in responsiveness with embryonic development. Pumping was induced by hatch water, crushed eggs of different ages, or crushed larvae. Nevertheless, the females were fairly sensitive to the chemical cue, as the lowest concentrations to induce significant responses were 10 larvae/ml of the hatch water and 0.05 crushed eggs/ml. The identity of the substance which induces pumping is currently under investigation.

Our initial question was whether the time of hatching is controlled by the female or the developing embryo. The time of hatching of a clutch depends upon those events which synchronized development of the embryos and those which control the actual hatching. An interaction between the eggs and female is responsible for synchronized development, while the embryo controls the actual event of hatching. The latter conclusion is supported by observations that the eggs hatched rhythmically independently of the female and upon hatching released a substance which induced pumping by the female. Pumping alone, however, did not cause hatching (Table II). Ennis (1973) similarly found that shaking the pleopods of the lobster *H. gammarus* did not induce egg hatching. Thus the conclusion reached by Pandian (1970) for this lobster, that the eggs control the actual event of hatching, also applied for *R. harrisii.*

The foregoing considerations allow us to speculate about the sequence of events during larval release. Some unknown aspect of maternal care is important in synchronizing embryo development. At the appropriate time the eggs are easily broken open. Initially, a few eggs hatch either by themselves or due to breakage by the female's normal body movements. A chemical cue is released which induces the female to move into position for larval release and to pump her abdomen. Pumping causes more eggs to hatch, which increases the concentration of the chemical cue,

TABLE II

Pumping response of crabs to clean water and a solution of homogenized eggs at concentrations of 20 eggs/ml (A) and 40 eggs/ml (B)

	Time before hatching (h)	# Pumps/2 min in clean water		# Larvae released in clean water	# Pumps/2 min in egg water		# Larvae released in egg water
		m	SE		m	SE	
A	−1.5	1.2	0.6	0	14	4.0	1
	−1	0.4	0.3	4	16.3	3.5	2
	−0.5	0.6	0.3	1	10.1	2.7	2
B	−1.0	0.75	0.5	0	10.5	5.2	0
	−0.5	2.25	0.75	0	19.0	9.5	0

The mean (m) number of pumps and the total number of larvae released in all experiments in the test bowls (clean water or egg homogenate) are shown. The number of crabs tested in A was 12 while 4 were tested in B. Tests were performed at 0.5 h intervals before the actual time of larval release, which occurred in the holding bowls shortly after the end of the day phase.

thereby causing further pumping. This sequence continues until all eggs which are ready have hatched.

A final consideration concerns the functional significance of having the actual time of hatching controlled by the embryo. Nocturnal larval release probably lowers mortality of larvae and adults due to predators which visually sight and actively pursue their prey (Ennis, 1975; Branford, 1978; DeCoursey, 1979; Bergin, 1981). Nevertheless, the female must expose herself at or near the entrance of her burrow during larval release making her still somewhat vulnerable to predation. By responding only when the appropriate chemical cue is present, the female does not try to release larvae at inappropriate times but rather concentrates her efforts on the times when the greatest number of larvae will be released. The consequence is synchronized hatching. In addition to inducing hatching, abdomen pumping also serves the function of oxygenating the embryos. If the female alone controlled larval release by regulation of pumping activity, then normal pumping during oxygenation could potentially release undeveloped embryos.

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